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Clinically Relevant Anaerobes

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Objectives

- Review appropriate specimens for anaerobic culture
- Identify best practices for specimen transport and culture
- Compare methods for identification of anaerobes
- Discuss susceptibility testing of anaerobes

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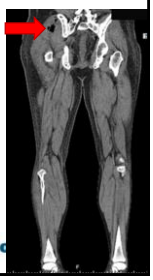
Clinical Presentations of Anaerobic Infections

- Abdominal abscesses
- Bacteremia
- Lemierre's syndrome (thrombophlebitis of the internal jugular vein)
  - Most commonly associated with *Fusobacterium necrophorum*
- Prosthetic joint infections
  - Most notably *Cutibacterium acnes*
- Cervicofacial infections/lumpy jaw
  - Most commonly associated with *Actinomyces*
- Gas gangrene/myonecrosis
- *Clostridioides difficile* infection (CDI)
  - Will not cover since infection is typically diagnosed by EIA or PCR



Taxonomy Update:

Anaerobic GPC formerly known as *Peptostreptococcus* species

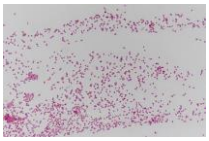
Former Name	Current Name
Peptostreptococcus prevotii, tetradius, vaginalis	*Anaerococcus* species
Peptostreptococcus parvulus/Streptococcus parvulus	*Atopobium parvulum*
Peptostreptococcus productus/Ruminococcus productus	*Blautia producta*
Peptostreptococcus magnus	*Finegoldia magna*
Peptostreptococcus banesae	*Gallicola barnesae*
Peptostreptococcus micros/Micromonas micros	*Parvimonas micra*
Peptostreptococcus asaccharolyticus, harei, indolicus	*Peptoniphilus* species
Peptostreptococcus heliotrinireducens	*Slackia heliotrinireducens*

- A few *Peptostreptococcus* species remain
  - Most notably – *Peptostreptococcus anaerobius*
- Biochemical methods do not provide an accurate identification
  - Must use MALDI or sequencing

Taxonomy Update:

Anaerobic Gram negative cocci

- Previously, any anaerobic GNC was identified as *Veillonella* spp.
- Other anaerobic GNC have been identified:
  - *Acidaminococcus* spp.
  - *Megasphaera* spp.
  - *Anaeroglobus* spp.
  - *Negativicoccus* spp.



- *Veillonella* spp. can be differentiated based on nitrate positivity
  - Other genera cannot be identified by biochemical methods
  - Must use MALDI or sequencing

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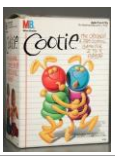
Taxonomy Update:

Gram positive rods

Former Name	New Name
Propionibacterium acnes	*Cutibacterium acnes*
Propionibacterium avidum	*Cutibacterium avidum*
Clostridium difficile	*Clostridioides difficile*



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Appropriate Specimens for Anaerobic Culture

Site	Acceptable Specimens	Unacceptable Specimens
Head and neck	Abscess aspirate Biopsy material	Throat or NP swabs Gingival swabs Surface material
Lungs	Biopsy material Bronchial brushing Material from percutaneous lung puncture Transtracheal aspirate Thoracotomy specimen	Sputum Tracheal aspirate Bronchoalveolar lavage
Central nervous system	Abscess aspirate Biopsy CSF	Aerobic swabs
Abdomen	Peritoneal fluid Abscess aspirate Bile Biopsy material	Aerobic swabs

Clinical Microbiology Procedures Handbook, 4th ed.

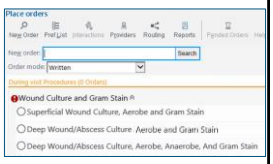
Appropriate Specimens for Anaerobic Culture

Site	Acceptable Specimens	Unacceptable Specimens
Urinary tract	Suprapubic aspirate	Voided urine Catheterized urine
Female genital tract	Culdoscopy specimens Endometrial aspirate Abscess aspirate Surgical biopsy IUD for *Actinomyces* species (controversial; correlate with pathology findings)	Vaginal or endocervical swabs
Bone and joint	Aspirate Biopsy material	Superficial material
Soft tissue	Deep aspirate (including surgical sinus collections) Biopsy material	Superficial material

Clinical Microbiology Procedures Handbook, 4th ed.

Ordering Anaerobic Cultures

- Use LIS/EMR order entry to your advantage
  - Configure systems to only allow anaerobic cultures on appropriate sources
  - Make it easy to order for appropriate sources
  - Make it difficult/impossible to order for inappropriate sources
- Example
  - CCMC's previous LIS/EMR listed Anaerobic Culture as a separate order



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Specimen Transport

- Specimens submitted for anaerobic culture should be transported as quickly as possible
  - With more hospital systems moving toward centralized labs, this is increasingly important
- Ensure ORs have transport media
- Educate clinicians on how to collect and transport specimens for anaerobic culture
  - Aspirates and tissues are preferred over swabs
  - If swabs are necessary, use flocked swabs in appropriate transport media

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Specimen Transport

Immediate Transport (≤30 min)

Delayed Transport

Culture Conditions – Incubation

- Type of incubation system depends on
  - Cost
  - Volume of anaerobic cultures
  - Space
- Incubation options include
  - Anaerobic incubator
  - Anaerobic pouches/boxes
  - Anoxomat

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Anaerobic Incubator/Chamber


Source: Anaerobe Systems

- Gas mixture: 85-90% N_2, 5% H_2, 5-10% CO_2
- Working with plates in incubator minimizes exposure to oxygen and maintains organism viability

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Pouch/Box System


- Water vs. water-free systems
- Water systems
  - Ampule crushed or water added to create anaerobic conditions
  - Absorb O_2 and generate H_2
- Water-free systems
  - Sachets absorb O_2 without generation of H_2
  - CO_2 levels may be higher than 10%

Anoxomat System (Advanced Instruments)

- Automated evacuation-replacement method
  - 3-5 minutes
- Gas mixture: 85% N_2, 5% H_2, 10% CO_2
- 0.16% residual oxygen content in the jar
  - Catalyst is added to remove residual oxygen
- 9" x 12" footprint

Culture Conditions – Media

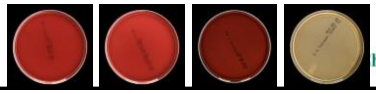
- Media should be reduced prior to inoculation
- Two options
  - Reduce media in-house (requires 24 h)
    - Track volumes to ensure appropriate amount of plates are reduced
  - Commercial pre-reduced anaerobically sterilized (PRAS) media
    - Oxygen-free packaging
    - Packaged in single packs, multipacks or combo packs

Culture Conditions – Media

- Oxyrase – enzyme added to media that reduces oxygen
- Oxyrase, Inc. produces OxyPRAS plates, which are PRAS plates that contain oxyrase
  - Maintain anaerobic environment on the benchtop for 2 hours prior to inoculation
- Also produces OxyDish – a self-contained environment

Culture Conditions – Media

- Solid media
  - Brucella blood agar, CDC blood agar or other enriched, nonselective media
  - Phenyethyl alcohol (PEA)
    - Aerobic and anaerobic Gram positive organisms and anaerobic Gram negative rods
  - Laked blood with kanamycin and vancomycin (LKV)
    - Gram negative rods
    - Not all anaerobic GNRs will grow → most notably *Fusobacterium* spp.
  - Bacteroides bile esculin agar (BBE)
    - *Bacteroides fragilis* group
    - *Bilophila wadsworthia*



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Culture Conditions – Media

- Additional selective agars
  - Fusobacterium selective agar (FSA)
  - Egg yolk agar (EYA)
    - *Fusobacterium* spp.
    - *Clostridium* spp.
- Broth media
  - Enriched thioglycolate
  - Chopped meat
- Anaerobic blood culture bottles
  - Blood cultures
    - Added benefit: many aerobes often grow faster in anaerobic bottles
  - Sterile body fluids

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Culture Conditions – Media

- What about CSF?
  - When is anaerobic culture appropriate for CSF?
    - Head injuries, head surgery, head and neck cancers, shunts
    - ENT infections: chronic otitis media and sinusitis, dental abscesses
    - GI disease
  - Many labs use thioglycolate broth as a catch-all for anaerobic bacteria
    - Utility is questionable – may cause more contamination/confusion than diagnosing true infections
  - One study showed an increased recovery of anaerobes by adding a PRAS Brucella blood agar plate to routine CSF cultures

J Clin Microbiol. 2014; 52(6):1824-9

Inoculation Consideration – Sonication of Prostheses

400 ml Ringer's solution

0.5 ml/plate

Trampuz A, Int Conf on Surg Infect, 2006

Culture Conditions – Incubation Length

- Incubation length
  - Incubate 5-7 days
  - Anaerobic blood agar should be held for the full incubation period
  - Selective agars may be discarded after 4 days
- Read plates after 24 or 48 hours
  - 24 h if using anaerobic chamber/incubator
  - 48 h for other methods

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Incubation Length – Special Considerations

- Requests (or sites where isolation is likely) for *Cutibacterium acnes* should be held 14 days
- Requests for *Actinomyces* should be held 10-14 days

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Examining Plates

- Minimize time plates are exposed to air
- As little as a 10 min exposure can kill some anaerobes
- Reduced media may oxidize quickly

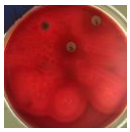
Workup of Anaerobic Cultures

- Workup practices vary by laboratory
- In many cases, knowing anaerobes are present is sufficient
  - However, important to identify potent/potentially resistant pathogens
    - *B. fragilis* group
    - *C. perfringens*
- **Always** compare growth to aerobic media
  - e.g. If three types of oropharyngeal flora are growing aerobically, and there is one true anaerobic GNR (such as *Prevotella*), include with normal flora, unless clearly predominant

Workup

- "Sterile" specimens
  - If <3 organisms present (aerobes + anaerobes), work up all organisms
  - If ≥3 organisms, check source in EMR
    - Source may not be "sterile" (e.g. tissue from decubitus ulcer)

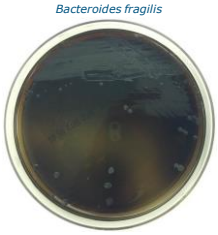
Workup



- Non-sterile specimens
  - ANA
    - Look for double zone of β-hemolysis (*C. perfringens*)
    - Look for predominant organism
    - Consider specimen source – what organism(s) may be important for the site?
  - BBE
    - Screen all growth for *Bacteroides fragilis* group (including *Parabacteroides* spp.)
    - *Bacteroides vulgatus* often forms clear colonies
  - PEA
    - Use for isolation of anaerobes if swarming *Proteus* or *Clostridium* is present
  - LKV
    - Previous guidelines stated to work up any growth, but workup should be based on source and growth on other media
  - Broth
    - Subculture only if no growth on plates and broth is turbid

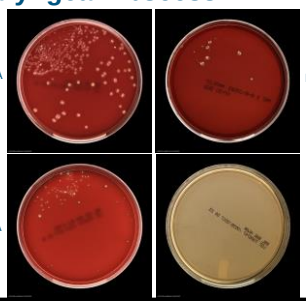
BBE

Bacteroides fragilis



Bacteroides vulgatus

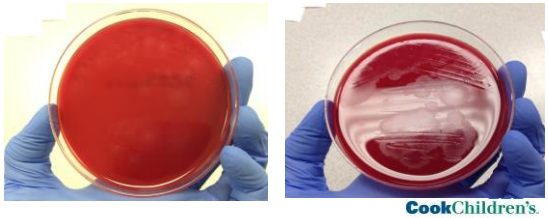
Retropharyngeal Abscess



Growth Characteristics

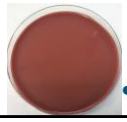
Colony Morphology	Possible Identification
"Bread crumb"	*Fusobacterium nucleatum*
"Fried egg"	*Fusobacterium necrophorum*, *Fusobacterium varium*
Double zone of beta-hemolysis	*Clostridium perfringens*
Fluorescence – brick red	*Porphyromonas* spp. (except *P. gingivalis*) pigmented *Prevotella* spp. *Veillonella* spp. *Eggerthella lenta*
Fluorescence – chartreuse (yellow-green)	*Fusobacterium* spp. *Clostridium difficile* *Clostridium innocuum*
Large with irregular margin/spread	*Clostridium* spp.
"Molar tooth"	*Actinomyces* spp.
Pigmentation – black or tan	*Porphyromonas* spp. Pigmented *Prevotella* spp.
Swarming	*Clostridium septicum* *Clostridium sordellii* *Clostridium tetani*

Swarming – *C. septicum*



Aerotolerance Test

- Must be performed on all isolates that meet workup guidelines
  - If using MALDI or sequencing, an aerotolerance test is unnecessary
- Use anaerobic blood agar and chocolate agar
  - Otherwise, may "identify" *Haemophilus*, *Aggregatibacter*, nutritionally variant streptococci, etc. as anaerobes



Special Potency Disks

Organism	Kanamycin 1,000 ug	Vancomycin 5 ug	Colistin 10 ug
Gram positive rods	V	S*	R
Gram positive cocci	V	R	S
Bacteroides fragilis group	R	R	R
Campylobacter (*Bacteroides*) *ureolyticus* group	S	R	S
Fusobacterium spp.	S	R	S
Porphyromonas spp.	R	S	R
Prevotella spp.	V	R	V
Veillonella spp.	S	R	S

*Except *Clostridium innocuum* and most *Lactobacillus* species (not *Lactobacillus acidophilus*)

Biochemical Identification

- Many biochemical methods are available for assistance with identification of anaerobes
- Some of the more commonly used biochemicals are:
  - Anaerobic catalase
  - Anaerobic indole
  - Egg yolk agar for lipase and lecithinase
  - Sodium polyanethol sulfonate (SPS) disks
    - Presumptive identification of *P. anaerobius*
  - Nitrate disks
    - Presumptive identification of *Veillonella* spp.
    - Nitrate disks can cause false-negative indole result

Overnight Biochemical System

- bioMérieux API 20A
- Must be incubated in an anaerobic environment
  - 24-48 hour incubation required, depending on isolate growth rate
- Metabolic reactions occur during incubation

Rapid Biochemical Systems

- Detect preformed enzymes
  - No. 3 or No. 4 McFarland required
- Incubated in aerobic environment
- 4 hour incubation
  - Beckman Coulter MicroScan Anaerobe panel
  - BD BBL Crystal Anaerobe ID Kit
  - bioMérieux Rapid ID 32A
  - Thermo Fisher Remel RapID ANA II
- 6 hour incubation
  - bioMerieux Vitek 2 ANC card

Biochemical Systems – Performance

- Correct identification to genus-level
  - Crystal Anaerobe ID: 75%
  - Vitek ANC: 71-79%
  - RapID ANA II: 81%
    - But had higher rate of misidentifications than Crystal and RapID ANA II
  - Rapid ID 32A: 87%
- Species-level identification rates: 50-60%

Anaerobe, 2010;16:355-61
J Clin Microbiol, 2011;49(5):1745-9
Anaerobe, 2014;30:126-8
Anaerobe, 2016;42:101-7

Limitations of Biochemical Methods

- Reactions can be difficult to interpret
- Databases are not routinely updated
- Cannot accurately identify anaerobic Gram positive cocci
- Issues with species-level identification of *Clostridium* spp. and *B. fragilis* group

Limitations of Biochemical Methods
CAP D-B Survey 2017

- Identification of *Propionibacterium granulosum*

Table 2. Identification Methods

Anaerobic method	No. of users	Correct genus and/or species (%)	*Propionibacterium granulosum* (%)	*Propionibacterium* sp. (%)	*Peptostreptococcus anaerobius* (%)
MALDI	281	93.6	57.7	35.9	0.7
Sequencing	15	93.4	66.7	26.7	0.0
Rapid ID 32A	18	72.2	61.1	11.1	11.1
BD BBL Crystal	17	64.7	52.9	11.8	0.0
API-20	34	52.9	14.7	38.2	2.9
Microscan	58	44.8	31.0	13.8	12.1
IDS Rapid ANA II	563	33.4	19.9	13.5	37.3
Biochemical	234	14.5	0.4	14.1	0.4
Other	98	7.1	1.0	6.1	7.1
Micro Media	38	5.3	0.0	5.3	0.0
Vitek 2	347	3.8	0.9	2.9	45.8

Limitations of Biochemical Methods

- Utilize your resources!
  - Gram stain to rule out GPC
  - Negative catalase would rule out *P. anaerobius*
- Subscribe to DEX (extended bacteriology) CAP survey even if you don't have MALDI or sequencing
  - Test your system – see what you get!

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16S Sequencing

- Gold standard for anaerobic identification
- Drawbacks
  - Relatively slow turnaround time
  - Technically challenging
  - Costly

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MALDI-TOF Mass Spectrometry

- Vitek MS
  - 92.5-99% to genus-level
  - 91.2% to species-level
- Bruker Microflex
  - 99% to genus-level
  - 85.8-99% to species-level

- MALDI is of the biggest advances in improving identification and turnaround times for anaerobes

Clin Microbiol Infect, 2014;20(4):335-9
Diagn Microbiol Infect Dis, 2014;79(2):144-8
Anaerobe, 2016;42:101-7
PLoS One, 2017;12(5):e0177929

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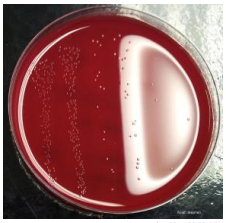
What to do with unusual identifications?

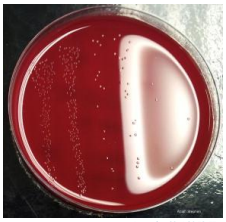
- Positive blood culture with Gram positive rods
  - "Regular" rods
  - Anaerobic growth only
- MALDI identification: *Solobacterium moorei*



Anaerobe, 2014;26:53-57

Solobacterium moorei??

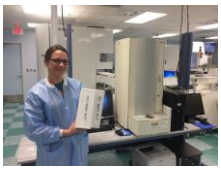
- Google? → www.bacterio.net → Pubmed
- Characteristics
  - Strict anaerobe
  - Non-sporeforming GPR
  - Catalase negative
- Associated with:
  - Halitosis
  - Bacteremia in immunocompromised hosts
- Our patient was an ED patient (discharged)
  - Reported name with "questionable significance" comment



Anaerobe, 2014;26:53-57

Other Unusual Identifications

- *Eggerthia catenaformis*
  - Formerly *Lactobacillus catenaformis*
- *Murdochiella asaccharolytica*
  - Anaerobic Gram positive coccus



Antimicrobial Resistance in Anaerobes

- *in vitro* resistance is increasing
  - Varies by country and region
  - Minimal outcomes (*in vivo*) data
- Clindamycin resistance increasing in all anaerobes
  - Resistance to other drugs varies by group or genus
- Most significant increases in resistance seen with *B. fragilis* group
  - All antimicrobials

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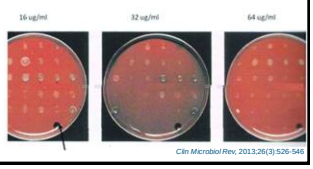
Susceptibility Testing – General

- Most anaerobic infections are polymicrobial
  - Presence of anaerobes drives treatment → Susceptibility testing is often unnecessary
  - Debridement is most important!
  - If requested, testing may be limited to the organism most likely to be resistant (e.g. *B. fragilis* group) (CLSI M100)
- Susceptibility testing may be useful for monomicrobial sterile site infections
  - Brain abscesses, joint infections, endocarditis, etc.
- Hospitals may consider monitoring local resistance patterns

Susceptibility Testing Methods

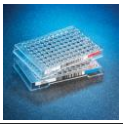
- Brucella blood agar or broth (supplemented with hemin, Vitamin K and sheep blood)
- Gold standard: agar dilution (42-48 h)

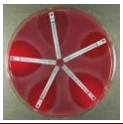


Clin Microbiol Rev, 2013;26(3):526-546

Susceptibility Testing Methods

- Broth microdilution (BMD) is only recommended for *B. fragilis* group members (46-48 h)
  - BMD should not be used for testing other anaerobes
- Gradient diffusion/Etests
  - Can overestimate metronidazole resistance if anaerobiosis is inadequate
  - May have unacceptable very major error (VME) rates for some antimicrobials





Ca
Clin Microbiol Rev, 2013;26(3):526-546
Diagn Microbiol Infect Dis, 1995;22(3):279-84
Diagn Microbiol Infect Dis, 1996;24:117-9

Susceptibility Testing Methods

- β-lactamase testing
  - Refer to package insert to see which genera are approved for testing
  - If performed and positive, report as resistant to penicillin and ampicillin
  - β-lactamase negative isolates may be resistant to penicillin and ampicillin by other mechanisms
    - If using a β-lactamase test, β-lactamase negative isolates should be confirmed

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CLSI M100 – Appendix D – Updated!

NOTE: Isolates collected from selected US hospitals from 1 January 2013 to 31 December 2016.[a]

D1. *Bacteroides fragilis* Group

Anaerobic Organisms	Number of Strains	Ampicillin-sulbactam		Number of Strains	Piperacillin-tazobactam		Number of Strains	Cefoxitin		Number of Strains	Ertapenem		Number of Strains	Imipenem		Number of Strains	Meropenem	
Percent susceptible (%S) and percent resistant (%R)[b]		%S	%R		%S	%R		%S	%R		%S	%R		%S	%R		%S	%R
Breakpoints, µg/mL		≤8/4	≥32/16		≤16/4	≥128/4		≤16	≥64		≤4	≥16		≤4	≥16		≤4	≥16
B. fragilis	129	84	2	1030	96	1	830	100	0	133	82	14	189	97	1	1505	93	5
B. thetaiotaomicron	76	82	5	252	87	0	208	13	64	–	–	–	70	100	0	328	99	0
B. ovatus	30	80	3	206	94	0	177	20	34	19[b]	84[b]	16[b]	49	100	0	236	95	1
B. vulgatus	20[b]	45[b]	15[b]	168	92	0	153	73	14	–	–	–	35	97	0	171	96	4
B. uniformis	19[b]	84[b]	0[b]	78	96	0	72	85	10	–	–	–	19[b]	100[b]	0[b]	93	100	0
Parabacteroides distasonis	27[b]	89[b]	19[b]	92	95	1	82	29	43	–	–	–	26[b]	100[b]	0	119	97	2
B. fragilis group without *B. fragilis*	172	74	8	796	91	0	742	36	36	19[b]	84[b]	16[b]	199	100	0	947	98	1
B. fragilis group (all 6 species listed)	301	78	5	1826	94	0	1572	70	17	152	82	14	388	98	0	2052	95	4

Source: CLSI M100S28E, 2018

CLSI M100 – Appendix D

NOTE: Isolates collected from selected US hospitals from 1 January 2013 to 31 December 2016.[a]

D2. Anaerobic Organisms Other Than *Bacteroides fragilis* Group

Anaerobic Organisms	Number of Strains	Ampicillin-sulbactam		Number of Strains	Piperacillin-tazobactam		Number of Strains	Imipenem		Number of Strains	Meropenem		Number of Strains	Penicillin	
Percent susceptible (%S) and percent resistant (%R)[b]		%S	%R		%S	%R		%S	%R		%S	%R		%S	%R
Breakpoints, µg/mL		≤8/4	≥32/16		≤32/4	≥128/4		≤4	≥16		≤4	≥16		≤0.5	≥2
Prevotella spp.	29[b]	97[b]	3[b]	63	100	0	29[b]	100	0	92	98	0	63	100	0
Fusobacterium spp.[c]	20[b]	100[b]	0[b]	55	96	2	75	95	4	20[b]	100[b]	0[b]	–	–	–
Anaerobic gram-positive cocci[d]	–	–	–	1853	99	1	134	99	0	1647	100	0	1647	100	0
Cutibacterium (formerly *Propionibacterium***)** *acnes*	–	–	–	18[b]	100[b]	0[b]	17[b]	94[b]	0[b]	–	–	–	–	–	–
Clostridium perfringens	15[b]	100[b]	0	410	100	0	23[b]	100[b]	0[b]	417	100	0	402	90	4
Clostridioides (formerly *Clostridium***)** *difficile*[e]	76	99	0	542	93	0	480	69	4	609	99	0	533	6	37
Other *Clostridium* spp.	–	–	–	439	94	1	71	99	0	390	100	0	390	69	13

Source: CLSI M100S28E, 2018

Take Home Points

- Specimen transport is extremely important
  - Improper transport can ruin a culture
- Consider the media and incubation conditions you use…help anaerobes grow!
- Time to identification is inversely proportional to clinical value
- MALDI is replacing biochemical methods for identification of anaerobes
- Susceptibility testing is often unnecessary
  - Use M100 as a resource

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Resources

- General
  - CLSI M56-A: Principles and Procedures for Detection of Anaerobes in Clinical Specimens
  - Clinical Micro Procedures Handbook, 4th ed.
  - Manual of Clinical Microbiology, 11th ed.
- Nomenclature/Taxonomy
  - www.bacterio.net
  - JCM Mini Review
    - *J Clin Microbiol*, 2017; 55:24–42
- Susceptibility Testing/Anaerobic Antibiogram
  - CLSI M100

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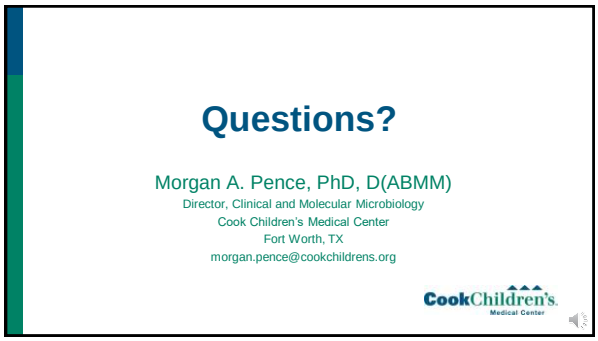
Questions?
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